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09/889586

Practitioner's Docket No. Lewis128519

CHAPTER II

Preliminary Classification:
Proposed Class:
Subclass:

TRANSMITTAL LETTER
TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/US00/40002	14 February 2000 (14.02.00)	14 February 1999 (14.02.99)
International Application Number	**International Filing Date**	**International Earliest Priority Date**

TITLE OF INVENTION: Deconvolving Far-Field Images Using Scanned Probe Data

APPLICANT(S): Lewis, Aaron

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. Section 371:

a. This express request to immediately begin national examination procedures (35 U.S.C. Section 371(f)).

b. The U.S. National Fee (35 U.S.C. Section 371(c)(1)) and other fees (37 C.F.R. Section 1.492) as indicated below:

2. Fees

CLAIMS FEE*	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALC-ULATIONS
	TOTAL CLAIMS	10 -20 =	0	x $18.00 =	$0.00
	INDEPEN-DENT CLAIMS	8 - 3 =	5	x $80.00 =	$400.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable) + $270.00				$270.00
BASIC FEE	U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in Section 1.482 has been paid on the international application to the U.S. PTO: and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(2) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. Section 1.492(a)(4)) $100.00				$100.00
	Total of above Calculations				= $770.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed. (note 37 CFR Sections 1.9, 1.27, 1.28)				- $385.00
	Subtotal				$385.00
	Total National Fee				$385.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. Section 1.21(h)). See attached "ASSIGNMENT COVER SHEET".				$40.00
TOTAL	Total Fees enclosed				$425.00

*See attached Preliminary Amendment Reducing the Number of Claims.

A check in the amount of $425.00 to cover the above fees is enclosed.

3. A copy of the International application as filed (35 U.S.C. Section 371(c)(2)) is not required, as the application was filed with the United States Receiving Office.

4. A translation of the International application into the English language (35 U.S.C. Section 371(c)(2)) is not required as the application was filed in English.

5. Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. Section 371(c)(3)) have not been transmitted. Applicant chose not to make amendments under PCT Article 19.

Date of mailing of Search Report (from form PCT/ISA/210): 25 April 2000.

6. A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. Section 371(c)(3)) has not been transmitted for reasons indicated in section 5.

7. A copy of the international examination report (PCT/IPEA/409) is not required as the application was filed with the United States Receiving Office.

8. Annex(es) to the international preliminary examination report is/are not required as the application was filed with the United Stated Receiving Office.

9. A translation of the annexes to the international preliminary examination report is not required as the annexes are in the English language.

10. An oath or declaration of the inventor (35 U.S.C. Section 371(c)(4)) complying with 35 U.S.C. Section 115 is submitted herewith, and such oath or declaration identifies the application and any amendments under PCT Article 19 that were transmitted as stated in Section 3 and/or 5; and states that they were reviewed by the inventor as required by 37 C.F.R. Section 1.70.

II. Other document(s) or information included:

11. An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a) is not required, as the application was searched by the United States International Searching Authority.

12. An Information Disclosure Statement under 37 C.F.R. Sections 1.97 and 1.98 is transmitted herewith.

Also transmitted herewith is/are Form PTO-1449 (PTO/SB/08A and 08B) and copies of citations listed.

13. An assignment document is transmitted herewith for recording.

14. Additional documents:

a. International Publication No. 00/47978

Specification, claims and drawing

15. The above items are being transmitted before 30 months from any claimed priority date.

AUTHORIZATION TO CHARGE ADDITIONAL FEES

The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No.: 10-1213

37 C.F.R. Section 1.492(a)(1), (2), (3), and (4) (filing fees)

37 C.F.R. Section 1.492(b), (c), and (d) (presentation of extra claims)

37 C.F.R. Section 1.17 (application processing fees)

37 C.F.R. Section 1.17(a)(1)-(5) (extension fees pursuant to Section 1.136(a))

Date: August 1, 2001

Signature of Practitioner

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2/PRTS

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JC18 Rec'd PCT/PTO 01 AUG 2001

DECONVOLVING FAR-FIELD IMAGES USING SCANNED PROBE DATA

I. FIELD OF THE INVENTION

The field of the invention is the combination of scanned probe microscopic data with far field optical and other images in order to deconvolve these images beyond the diffraction limit.

II. BACKGROUND OF THE INVENTION

Lens based far-field imaging is limited in the resolution that it can achieve by the characteristics of the lens. In general, there are problems of diffraction of the lens, problems with aberration of the lens and problems of out-of-focus radiation. The latter, out-of-focus radiation problem is generally partially improved by the use of confocal imaging methodologies; in optics, non-linear imaging techniques are also useful The solution of the former diffraction and aberration problems is partially addressed by measuring the point spread function of the lens and then using computer deconvolution to remove these effects from the image. Even the latter out-of-focus problem can be addressed without confocal or non-linear imaging by considering both the in-focus and the out-of-focus point spread function and using deconvolution routines to try and eliminate these effects. Numerous algorithms have been devised to address these problems of computer deconvolution of far-field imaging data, but none are completely successful and none of them have the ability to

carry the far-field image to the realm beyond the diffraction limit as defined, for example, by the Rayleigh criterion, which is approximately ½ of the wavelength of the radiation that is being used. For visible 500 nm light this is 250 nm.

In terms of deconvolution algorithms, a powerful mathematical approach is based on the use of constraints. For example, in deconvolving a far-field image a good constraint would be to define with high precision the cell membrane of a cell that is stained with a dye and is being imaged by a lens. By precisely defining the position of a cell membrane or a portion of the cell membrane, it is possible precisely to define where the staining in the image is confined and beyond which point or points there is no staining and its associated optical phenomenon. Such a constraint would give many deconvolution algorithms a powerful advantage. Nonetheless, even though the idea is mathematically a powerful concept [Carrington et al, Science 268, 1483 (1995)], it is seriously limited in far-field optics by the inability to obtain a constraint that is better than the optical resolution.

III. STATE OF PRIOR ART

No one has previously attempted to incorporate near-field optical data and other scanned probe microscope data such as that which is obtained from atomic force microscopy to the problem of providing constraints in the deconvolution of far-field optical and other far-field imaging techniques.

IV. SUMMARY OF THE INVENTION

In accordance with the present invention, a method for deconvolving far-field optical images beyond the diffraction limit includes the use of near-field optical and other scanned probe imaging data to provide powerful and new constraints for the deconvolution of far-field data sets. Near-field data, such as that which can be obtained from atomic force microscopy on a region of the far-field data set in an integrated and inter-digitate way, is used to produce resolutions beyond the diffraction limit of the lens that is being used. In the case of non-linear optical imaging or other microscopies, resolutions beyond that which is achievable with these microscopies can be obtained.

V. BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing, and additional objects, features and advantages of the present invention will become apparent to those of skill in the art from the following detailed description of a preferred embodiment thereof, taken with the accompanying drawings, in which:

Fig. 1A illustrates a first image of a model object, a second image of the object imaged by a lens having a known point spread function, a third, or sampled image recorded by a CCD device, and a fourth image of a deconvolution of the sampled image without constraints;

Fig. 1B is a graphical illustration of the deconvolution of Fig. 1A;

Fig. 2A illustrates the same images as Fig. 1A, wherein the deconvolution is performed with constraints obtained from near-field imaging data; and

Fig. 2B is a graphical illustration of the deconvolution of Fig. 2A.

VI. DESCRIPTION OF THE INVENTION

The present invention incorporates data that has never been incorporated previously to resolve issues and problems in far-field imaging. This data comes from near-field optical microscopy and its scanned probe cousins, such as atomic force imaging (AFM), and presents the far-field microscopist with constraints that will dramatically improve the far-field imaging of all forms of far-field microscopy. These improvements are available for both linear and non-linear optical microscopy and even those microscopies that use particles rather than electromagnetic radiation. The invention also allows for using one form of scanned probe microscopy to deconvolve another, to thereby improve the resolution of a scanned probe microscope before this data is used in the present invention, as described below.

In accordance with the invention, it is essential to obtain near-field optical or other scanned probe imaging data in a way that is fully integrated with a far-field data set that is to be improved. In one embodiment of this invention in which far-field optical microscopic data is to be deconvolved, one useful approach to achieving the required full integration of the data sets is to use a charge coupled device (CCD) to record the far-field data that corresponds to an associated scanned probe pixel. Furthermore, it is additionally useful if the scanned probe data sets that are to be used in the deconvolution are obtained in simultaneous channels. This can be done, for example, with a tip that is multifunctional such as a tip that is both a subwavelength light source and an AFM sensor [K. Lieberman, et al., Rev. Sci. Instr. 67, 3567 (1996)] that can be used in contact or near-contact with a surface of a

specimen that is being imaged.

However, even before the far and near-field imaging process is begun, lens images of a subwavelength light source of known dimension are recorded on a CCD. These images, both in-focus and out-of-focus, are used to obtain a measure of the lens point spread function (PSF), even with perturbation of the object, or sample, being imaged. This PSF is the lens function that convolutes with the functional representation of a sample to give the blurred image that is the far-field image with its associated diffraction and other problems mentioned in the Background section, above. Alternately, the convolution effect of the lens can also be determined if a known high resolution sample is imaged and the error between the real and the ideal image is represented as a blurring function introduced by the lens. Obviously, if the imaging task is fluorescence then the high resolution test object will have to be similarly fluorescent. Thus, the first task in this method is to determine the PSF of the lens that is to be used to image the desired object, or sample.

The next step is to record with the CCD the far-field image of the object. Subsequently, super-resolution optical data is recorded for specified points on the object surface. An example of such data can include defining an exact point at which the optical contrast in an object terminates; i.e., defining the edge of an object to much better than the optical resolution if the far-field image is an optical image or defining the x, y and z point, or voxel, at which there is a contrast change, and relating this point to another point of contrast change in the sample. The two points

of contrast change in the sample could, for example, be at different planes as defined by the lens in the far-field, and the near-field scanned probe data, obtained through a multifunctional AFM sensor tip and simultaneously recorded, could provide not only the x-y separation of the two points but also the Z separation at a resolution that is better than any optical approach such as confocal microscopy.

The exemplary constraints listed above, or for that matter any constraints from scanned probe technology, have never been used in this cross-fertilization mode with far-field optical microscopy or, for that matter, any far-field microscopic technique. In addition, such cross-fertilization has not been used between scanned probe techniques, i.e. to use near-field optical data as a constraint at deconvolving near-field atomic force microscopy data, or the reverse. With regard to this latter mode of deconvolution there is quite a bit of synergism between, for example, the near-field optical and the near-field AFM since the functional dependence of the decay of the effect, as a function of probe sample separation, is near-exponential for the near-field optical and occurs over a much shorter distance for the simultaneously recorded AFM technique. In essence, then, the scanned probe microscopy synergism can first be used to improve the near-field scanned probe microscopy data and then that data can be applied as a constraint to the far-field microscopy deconvolution in question. At this point, it is also important to note that the order of the procedures listed in this section is not a critical part of the invention; any combination of the order of the steps or partial combination of steps constitutes this invention. For example, if the near-field optical data is not used to deconvolve to higher resolution the atomic force

microscopy data, it, and the atomic force microscopy data could still be used to deconvolve the far-field data.

The CCD mentioned above is a most useful method to obtain the digitized image of the far-field, but this can also be accomplished with confocal microscopy. In the case of far-field optical microscopy it should be noted that there could be innovative ways to record the confocal data without any confocal aperture. For example, a film of material that produces a non-linear optical signal known as second harmonic generation (SHG) can be used as part of this invention to record a confocal image. In this case the light from the plane of focus is focused by the lens onto the film, such as a plastic film of purple membrane that produces SHG [Z. Chen et al., Applied Optics 30, 5188 (1991)], with an intensity that is higher than from any other plane in the sample that is being focused by the lens. A film that would produce a second harmonic signal only where there is a point of light from the plane of focus in the sample could be used to replace the confocal pinhole. Such a film would act as a parallel filter for light from the plane of focus in the sample. This could be used together with an appropriate filter after the film to remove the fundamental wavelength that was illuminating the sample and to pass only the SHG to the detector which could be a CCD rather than the single channel detector that is normally part of a confocal set-up. This could be done with SHG or other non-linear optically active films.

VII. ADVANTAGES OVER PRIOR ART

Scanned probe microscopy data has not been used as a constraint in mathematical constraint algorithms to deconvolve far-field optical images. In addition, the use from multi functional scanned probe microscopy of one parameter, such as near-field optical data, to deconvolve another parameter such as atomic force microscopy data has also not been applied. The advantage over prior art arises from the increase in spatial resolution that this approach achieves.

VIII. APPLICATIONS

Methodologies for increased spatial resolution always open new doors in science and technology, as evidenced by the revolution that was caused by the introduction of the electron microscope.

To test the essence of this invention a calculation has been performed on a model far-field optical data set, as illustrated in Figs. 1A, 1B, 2A and 2B, to which reference is now made. In Figure 1A there are four images, one in each of four horizontal rows. At the right-hand end of each row is an intensity legend for comparison. Starting from the top of Fig. 1A, the first row of the figure represents a model object which has a dimension that cannot be resolved optically; for example, an object having a dimension of about 0.2 micron. As may be seen in the first row, the object has a first sharp light-to-dark transition point at its left-hand edge, a second, dark-to light transition near the center, a third, light-to-dark transition to the right of the second transition, and a fourth, dark-to-light transition at the left-hand

edge of the model object. When the object is imaged by a lens with a known point spread function, each of the transition points on the object is blurred because the dimensions are too small to be resolved, and the blurred image, which is the second row from the top in Figure 1, results. When this image is recorded by a CCD there is further blurring due to the pixel character of the CCD, as illustrated in the third row. The last image, row 4 in this figure, is produced by processing the image of row 3 with a standard deconvolution algorithm without the imposition of the type of constraints that are central to this invention, in which a new approach to provide constraints is described. Figure 1B is a chart illustrating the intensity variations produced by the model object and by the restored (deconvoluted) object.

In Figure 2A the same object, lens, and CCD are used, and rows 1-4 illustrate the same object and images as described above with respect to Fig, 1A, except that in the deconvolution algorithm four points are given the resolution of near-field optics. These points are, in going from left to right in the model object illustrated in the top row of Fig. 2A, the first, second, third and fourth alterations in contrast, as described above with respect to Fig. 1A. The results of using such constraints is seen in the vastly improved quality of the deconvolved image in the bottom row of that Figure.

Although the invention has been described in terms of preferred embodiments, it will be understood that numerous variations and modifications may be made without departing from the true spirit and scope thereof as set forth in the following claims.

What is claimed is:

1. A method for deconvolving far-field optical microscopic images to a level of resolution that has never been achieved previously by introducing scanned probe microscopy data in an interdigitated, integrated fashion with confocal or charge couple device imaging fashion such that every pixel or voxel from the super-resolution scanned probe microscopy data set is directly related to an image pixel or voxel in the far-field data set and then using the scanned probe data as a mathematical constraint to deconvolve the far-field image.

2. A method that uses multifunctional scanned probe microscopy data so that one scanned probe microscopy parameter can be deconvolved by integrating data from another such as, but not excluding other combinations, deconvolving an atomic force microscopy image with a near-field optical microscopy image or vice versa.

3. The method as in claim 1 in which the procedure in claim 2 is used in combination with the method in claim 1.

4. A method to obtain the point spread function, by using a known high resolution sample either fluorescent or non-fluorescent, which is imaged and the error between the real and the ideal image is represented as a blurring function introduced by the lens and is representative of an accurate point spread function.

5. A method to obtain a digitized image of the optical far-field in addition to simple

confocal or charge coupled device imaging that can be used for integrated near-field and far-field imaging that uses a film of material that can produce a non-linear optical signal known as second harmonic generation in which light from the plane of focus of the lens would be focused by the far-field lens onto a film, such as plastic purple membrane films, with an intensity that is higher than from any other plane in the sample that is being focused by the lens and thus such a film could be used to replace the confocal pinhole in confocal imaging by using a film that would light up only where there is a point of light from the plane of focus in the sample and thus such a film would act as a parallel filter for light from the plane of focus in the sample with this light being imaged through a filter for the fundamental illuminating frequency onto a charge coupled device.

6. A device that collects in an integrated, correlatable fashion that as a result allows for deconvolving far-field optical microscopic images to a level of resolution that has never been achieved previously by introducing scanned probe microscopy data in an interdigitated, integrated fashion with confocal or charge couple device imaging such that every pixel or voxel from the super-resolution scanned probe microscopy data set is directly related to an image pixel or voxel in the far-field data set and then using the scanned probe data as a mathematical constraint to deconvolve the far-field image.

7. A device that collects in an appropriate integrated fashion multifunctional scanned probe microscopy data so that one scanned probe microscopy parameter can be

deconvolved by integrating data from another such as, but not excluding other combinations, deconvolving an atomic force microscopy image with a near-field optical microscopy image or vice versa.

8. A device as in claim 5 that also has the capabilities of claim 6.

9. A device to obtain the point spread function, by using a known high resolution sample either fluorescent or non-fluorescent, which is imaged and the error between the real and the ideal image is represented as a blurring function introduced by the lens and is representative of an accurate point spread function.

10. A device to obtain a digitized image of the optical far-field in addition to simple confocal or charge coupled device imaging that can be used for integrated near-field and far-field imaging that uses a film of material that can produce a non-linear optical signal known as second harmonic generation in which light from the plane of focus of the lens would be focused by the far-field lens onto a film such as plastic purple membrane films, with an intensity that is higher than from any other plane in the sample that is being focused by the lens and thus such a film could be used to replace the confocal pinhole in confocal imaging by using a film that would light up only where there is a point of light from the plane of focus in the sample and thus such a film would act as a parallel filter for light from the plane of focus in the sample with this light being imaged through a filter for the fundamental illuminating frequency onto a charge coupled device.

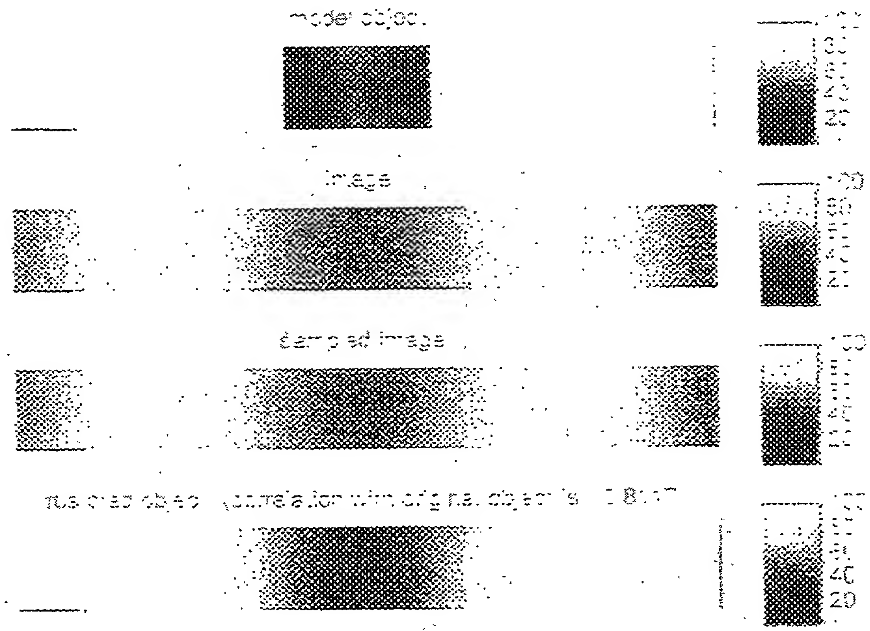
model object
image
damped image
20
40

FIG. 1A

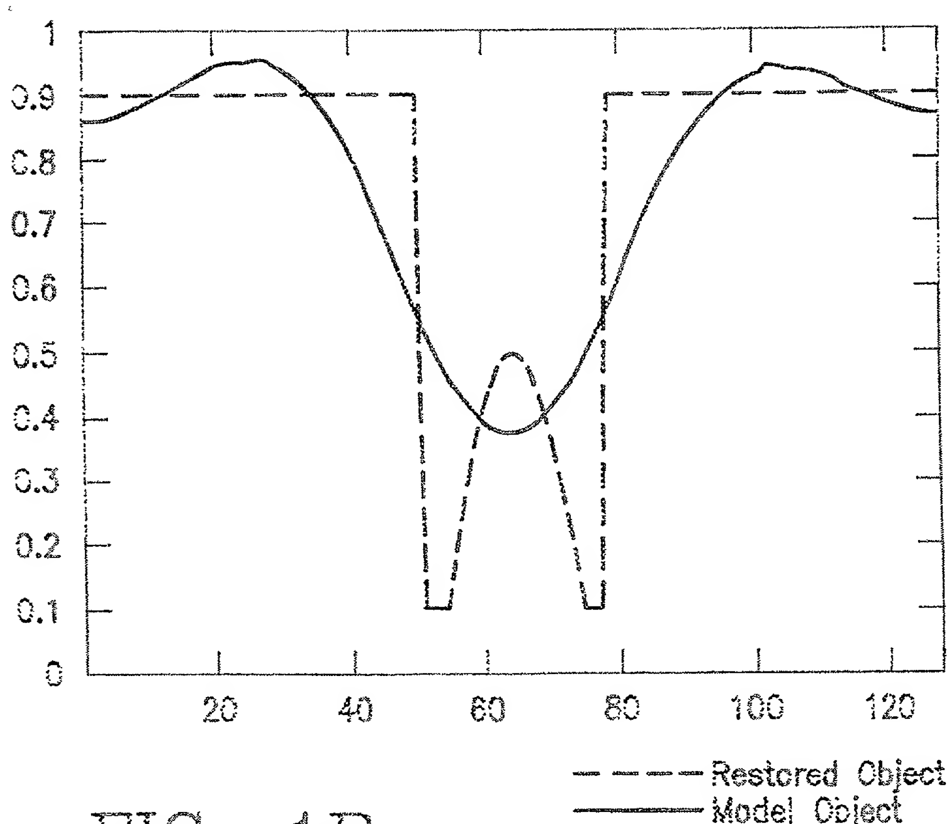
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
100
120
Restored Object
Model Object

FIG. 1B

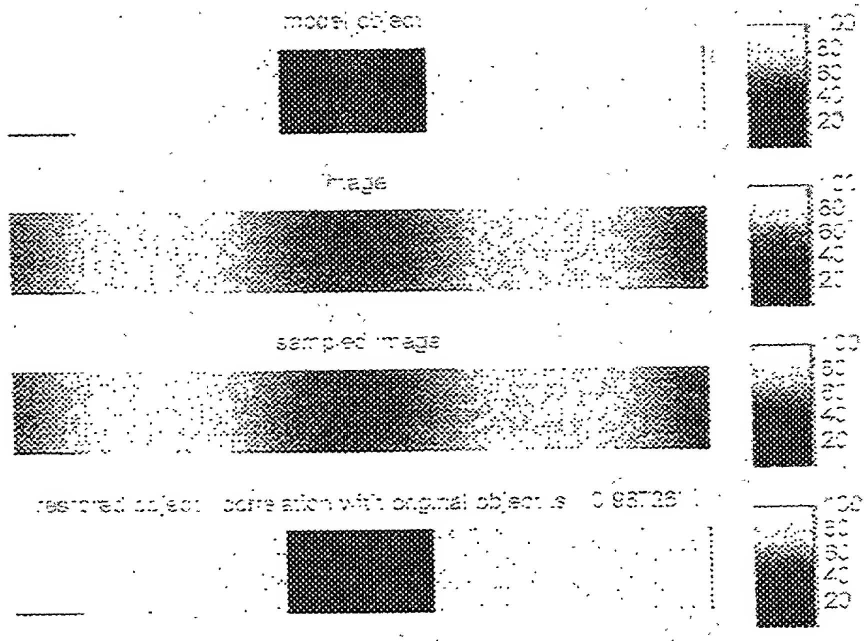
model object
image
sampled image
restored object
20
40
60
80
100

FIG. 2A

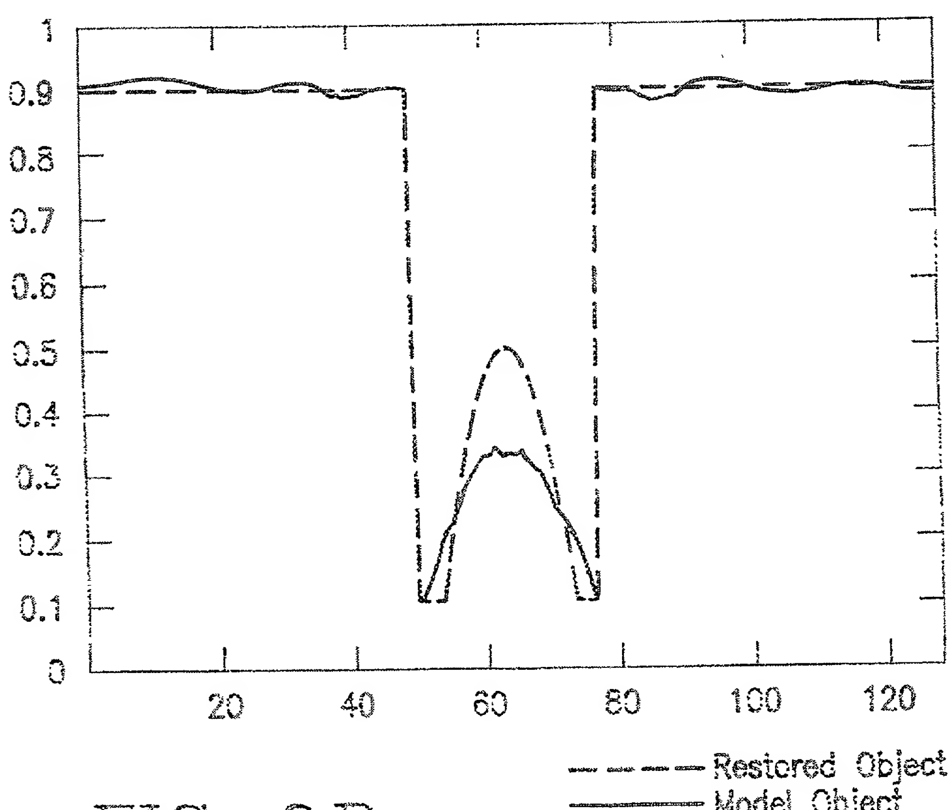
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
100
120
Restored Object
Model Object

FIG. 2B

Practitioner's Docket No. Lewis128519 ***PATENT***

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is for a national stage of PCT application.

INVENTORSHIP IDENTIFICATION

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

Deconvolving Far-Field Images Using Scanned Probe Data

SPECIFICATION IDENTIFICATION

The specification was described and claimed in PCT International Application No. PCT/US00/40002 filed on February 14, 2000.

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, Section 1.56, and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 C.F.R. Section 1.98.

PRIORITY CLAIM (35 U.S.C. Section 119(a)-(d))

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any

foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Such applications have been filed as follows.

PRIOR PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. SECTION 119(a)-(d)

INDICATE IF PCT	APPLICATION NUMBER	DATE OF FILING DAY, MONTH, YEAR	PRIORITY CLAIMED UNDER 35 U.S.C. SECTION 119
PCT	PCT/US00/40002	14 February 2000	yes

PRIOR FOREIGN APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. SECTION 119(a)-(d)

COUNTRY	APPLICATION NUMBER	DATE OF FILING DAY, MONTH, YEAR	PRIORITY CLAIMED UNDER 35 U.S.C. SECTION 119
Israel	128519	14 February 1999	yes

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Eric S. Spector	22495
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Colin D. Barnitz	35061
Jennifer P. Yancy	47003
Kenneth M. Jones	48203

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I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

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George M. Cooper
703-415-1500

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

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